Reactivating Fetal Hemoglobin Expression in Human Adult Erythroblasts Through BCL11A Knockdown Using Targeted Endonucleases.

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Public Summary:
We examined the efficiency, specificity, and off-target mutation rates of three different genome editing tools: zinc finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 systems. Our results indicated that the ZFNs were best at making the targeted gene editing mutation, and had the least off-target mutations generated compared to TALENs and CRISPER/Cas9 systems.

Scientific Abstract:
We examined the efficiency, specificity, and mutational signatures of zinc finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 systems designed to target the gene encoding the transcriptional repressor BCL11A, in human K562 cells and human CD34(+) progenitor cells. ZFNs and TALENs were delivered as in vitro transcribed mRNA through electroporation; CRISPR/Cas9 was codelivered by Cas9 mRNA with plasmid-encoded guideRNA (gRNA) (pU6.g1) or in vitro transcribed gRNA (gR.1). Analyses of efficacy revealed that for these specific reagents and the delivery methods used, the ZFNs gave rise to more allelic disruption in the targeted locus compared to the TALENs and CRISPR/Cas9, which was associated with increased levels of fetal hemoglobin in erythroid cells produced in vitro from nuclease-treated CD34(+) cells. Genome-wide analysis to evaluate the specificity of the nucleases revealed high specificity of this specific ZFN to the target site, while specific TALENs and CRISPRs evaluated showed off-target cleavage activity. ZFN gene-edited CD34(+) cells had the capacity to engraft in NOD-Prkdc(SCID)-IL2Rgamma(null) mice, while retaining multi-lineage potential, in contrast to TALEN gene-edited CD34(+) cells. CRISPR engraftment levels mirrored the increased relative plasmid-mediated toxicity of pU6.g1/Cas9 in hematopoietic stem/progenitor cells (HSPCs), highlighting the value for the further improvements of CRISPR/Cas9 delivery in primary human HSPCs.